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Infusion of Select Leukemia-Reactive TCR Vβ$^+$ T Cells Provides Graft-Versus-Leukemia Responses With Minimization of Graft-Versus-Host Disease Following Murine Hematopoietic Stem Cell Transplantation

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ABSTRACT

T-cell receptor (TCR) Vβ–expression analysis by complementarity-determining region 3 (CDR3)-size spectratyping can identify the reactive populations in an immunologic response. This analysis was used in this study to characterize the Vβ responses of C57BL/6 (B6) CD4$^+$ and CD8$^+$ T cells directed to either alloantigen (against [B6×DBA/2]F1; anti-H2^d) or the syngeneic myeloid leukemia MMB3.19. Vβ families exhibiting reactivity to the leukemia cells were then enriched for and administered in both syngeneic and allogeneic hematopoietic stem cell transplantation (HSCT) models to assess in vivo graft-versus-leukemia (GVL) potential. In syngeneic transplants, enrichment for pools of selected Vβ families (Vβ7, -11, and -13) of T cells or for a single Vβ family (Vβ7) of CD4$^+$ T cells conveyed a beneficial GVL response to the recipients. Furthermore, in the haploidentical allogeneic model, both Vβ6,7-enriched donor B6 T cells and Vβ7-enriched CD4$^+$ T cells exhibited significant GVL responses with concomitant minimization of graft-versus-host disease (GVHD) development compared with equal numbers of unfractionated T cells. These results suggest that CDR3-size spectratype analysis of and subsequent selection from donor T-cell repertoires can be an effective approach to separate GVL and GVHD potential following allogeneic HSCT.

KEY WORDS

Graft-versus-host disease • T-cell repertoire • T cell–receptor Vβ families

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) has been used to treat several forms of leukemia and has been particularly effective in increasing the survival rates of patients with acute myelogenous leukemia and chronic myelogenous leukemia [1,2]. Two inversely related complications, however, graft-versus-host disease (GVHD) and leukemic relapse, have high impact on the morbidity and mortality rates following HSCT [3-8]. A positive clinical outcome largely depends on balancing the negative effects of GVHD and the positive effects of graft-versus-leukemia (GVL) activity of donor T cells. When T cells are depleted from stem cell allografts, there is a marked reduction in GVHD but there is an increased incidence of leukemic relapse [3-8]. Donor T cells are recognized as the only means of avoiding leukemic relapse in chronic myelogenous leukemia [9], and delayed infusion of T cells has become a standard treatment approach [10-14], although the success of this treatment is still significantly hampered by the incidence of GVHD. Therefore, a major goal of donor T-cell therapy is to provide a GVL effect while lessening the incidence and severity of GVHD.

Various strategies have been employed to separate GVHD from GVL activities, including CD4$^+$, CD6$^+$, and CD8$^+$ T cell–subset depletions [15-17]; in vivo α-CD3 monoclonal antibody (MoAb) administration [18,19]; in vivo cytokine manipulation [20,21]; ex vivo tolerization of alloreactive T cells [22,23]; and separation of donor T cells based on functional phenotypes (cytokine profiles [24] or cytolytic effector mechansims [25-27]). These approaches, however, do not take into account the antileukemia specificity of donor T cells but rely instead upon broad differences

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in activation thresholds or functional effector mechanisms for a selective antileukemia effect.

An approach that should theoretically allow for a more specific separation of GVH-reactivity from GVL effects involves analysis of T-cell receptor (TCR) Vβ chain utilization in these responses by complementarity-determining region 3 (CDR3)-size spectratyping and selective administration of those Vβ families demonstrating an antileukemic response. TCR Vβ CDR3-size spectratyping is a method for identifying the reactivity of T-cell populations responding to given antigen challenges [28] such as major histocompatibility complex (MHC) or minor histocompatibility alloantigen differences or leukemia-related antigens. This latter category would include leukemia-specific antigens such as bcr-abl [29-31] and overexpressed or aberrantly expressed normal antigens such as proteinase 3 [32]. It is important to note that separation on the basis of T-cell reactivity can be performed without having to define the antigens being recognized and would allow for a more specific administration of leukemia-reactive T-cell populations.

Vβ CDR3-size spectratyping has been used to identify alloreactive populations of T cells in murine [33] and human [28] transplantation systems, and it has been recently demonstrated that depletion of donor cells based on spectratype skewing can allow for minimization of CD4-mediated GVHD in an MHC-matched allogeneic murine model [34]. However, the hypothesis that this approach could allow for a meaningful GVL response with a concurrent GVHD reduction has yet to be directly supported. To address this possibility, we analyzed the syngeneic anti–myeloid leukemia responses for both $CD4^+$ and $CD8^+$ T cells in the C57BL/6 (B6) anti-MMB3.19 model and the alloreactive responses in the B6 anti-(B6×DBA/2)F1 (B6D2) strain combination, using Vβ CDR3-size spectratyping. We report here that infusion of leukemia-reactive Vβ populations of T cells in either syngeneic or allogeneic bone marrow transplantation (BMT) settings allowed for a significant retention of GVL activity, and, in the allogeneic setting, a concomitant mitigation of GVHD.

MATERIALS AND METHODS

Mice

Male B6 ($H2^b$) and B6D2 ($H2^{b/d}$) mice were purchased from the National Cancer Institute Animal Procurement Program (Frederick, MD). Donor mice were aged 8-10 weeks and recipients aged 6-10 weeks. Mice were housed in a sterile environment in microisolators and given autoclaved food and acidified water ad libitum.

Cell Line and Media

MMB3.19 is a *c-myc* retrovirus–transformed myeloid leukemia line of B6 origin that has been previously characterized and does not produce retrovirus particles [17,27,35]. MMB3.19 was grown in RPMI 1640 (Mediatech, Herndon, VA) plus 10% fetal calf serum (Sigma Chemical, St Louis, MO) supplemented with L-glutamine, penicillin/streptomycin (Mediatech) and 2-mercaptoethanol (Life Technologies, Grand Island, NY), at 37°C in 7% CO_2. Phosphate buffered saline (PBS) supplemented with 0.1% bovine serum albumin (BSA; Sigma) was used for in vitro manipulation of donor bone marrow cells and lymphocytes. Cells were resuspended in PBS alone for all injections into recipients.

Monoclonal Antibodies

Anti-Thy-1.2 (J1j; rat immunoglobulin [Ig]M [36]), anti-CD8 (3.168; rat IgM [37]), and anti-CD4 (RL172; rat IgM [38]) MoAbs were obtained from ascites fluid and used with guinea pig C′ (C′) (Rockland, Boyertown, PA) for cell depletions (always used at 1:10 dilution). Affinity-purified goat anti-mouse IgG antibody (Cappel-Organon Teknika, West Chester, PA) was used for B-cell panning. For phenotypic analysis or selection of donor T-cell populations, fluorescein isothiocyanate (FITC)-labeled MoAbs were used, directed to the following determinants: CD3, CD4, CD8, B220, Vβ2, -3 -4, -5.1/5.2, -6, -7, -8.1/8.2, -8.3, -9, -11, -12, -13, and -14 (all from Pharmingen, San Diego, CA).

Preparation of Cells

Anti-Thy-1 MoAb–treated (T cell–depleted) bone marrow (ATBM) was prepared by flushing femurs and tibiae of donor mice and incubating the bone marrow cells with J1j MoAb (1:200 dilution) and C′ for 45 minutes at 37°C. To obtain T cell–enriched donor populations, spleen and lymph node (LN) cells were treated with red-blood-cell lysing solution (containing 0.8% NH_4Cl) and allowed to adhere for 1 hour at room temperature to plastic Petri dishes coated with a 1:200 dilution of goat anti-mouse IgG (to remove B cells). The collected nonadherent T cells were further enriched for either $CD4^+$ (by treatment with anti-CD8 MoAb at 1:100 dilution and C′) or $CD8^+$ T cells (by treatment with anti-CD4 MoAb at 1:100 dilution and C′) for 45 minutes at 37°C. Vβ-enriched or depleted T-cell populations were obtained by magnetic bead separation using the varioMacs system (Miltenyi Biotec, Bergisch Gladbach, Germany).

Flow Cytometry

For phenotypic analysis of T cell–enriched populations, appropriate FITC-labeled MoAbs in 25 μL were incubated with 2×10^5 cells in 96-well round-bottom plates for 30 minutes at 4°C and washed 3 times with 100 μL PBS plus 1% BSA plus 0.02% sodium azide. Samples were then fixed with 1% paraformaldehyde and analyzed for fluorescence on an EPICS Elite ESP analyzer (Coulter Electronics, Hialeah, FL) in the Kimmel Cancer Center Flow Cytometry Facility.

Preparation of RNA and Complementary DNA

Alloreactive $CD4^+$ and $CD8^+$ T cells were isolated by B-cell panning and appropriate MoAb plus C′ treatment (as described above) from spleens of irradiated (split-dose, 13 Gy) B6D2 recipients 5 days after transplantation of 3×10^7 B6 T cells. Anti-MMB3.19 $CD4^+$ and $CD8^+$ T cells were isolated from popliteal LN of B6 mice primed (week –3) and boosted (week –1) with 2×10^6 irradiated (40 Gy) MMB3.19 cells injected subcutaneously into the footpad. Control B6 $CD4^+$ and $CD8^+$ T cells were obtained from LN of naive B6 mice. Total cellular RNA from these T-cell populations was generated by homogenation in 0.5 to 1 mL of Ultraspec (Biotecx Laboratories, Houston, TX), followed by addition of chloroform (1:5 volume), shaking, incubation on ice for 5 minutes, and centrifugation for 15 minutes at 14,000 rpm. The aqueous phase was transferred to an Eppendorf tube,

and RNA was precipitated with isopropanol (1:1 volume) for 10 minutes at 4°C followed by centrifugation for 20 minutes at 4°C. After washing 2 times with 75% ethanol in diethyl pyrocarbonate (DEPC) water, the RNA pellet was resuspended in 25 μL DEPC water and stored at –20°C. Recovery of RNA was determined by spectrophotometry. Oligo (dT) was used as a primer for reverse transcription of RNA to complementary (c) DNA. Total RNA of 2 μg in a volume of 9.5 μL was heated to 70°C and added to a master mix (17.5 μL) of 1 μL RNasin (40 U/μL); 6 μL 5× Maloney murine leukemia virus (MMLV) reverse transcriptase reaction buffer; 6 μL oligo (dT) primer (20 mmol/L); 1.5 μL deoxynucleotide triphosphates A, G, C, and T (25 mmol/L each); and 3 μL MMLV reverse transcriptase (300 U/μL), incubated at 37°C for 1.5 hours followed by a 3-minute incubation at 95°C, and stored at –20°C. All reverse transcriptase reagents were purchased from Promega (Madison, WI).

Polymerase Chain Reaction Amplification and CDR3-Size Spectratyping

Polymerase chain reaction (PCR) of T-cell cDNA samples was performed using 1.2 μL cDNA (50 ng/μL); 1.5 μL 10× AmpliTaq Gold polymerase buffer; 0.12 μL AmpliTaq Gold polymerase; 1.5 μL of deoxynucleotide triphosphates A, G, C, and T (25 mmol/L each); 1.5 μL $MgCl_2$; 5.18 μL DEPC treated water; and 1 μL each of fluorescence-labeled β2m antisense primer, unlabeled β2m sense primer, fluorescence-labeled constant primer (Cβ), and unlabeled Vβ-specific primers for each Vβ family. All primers used have been previously described [33,39]. All PCR reagents were purchased from Perkin-Elmer (Emeryville, CA). For the MMB3.19-presensitized samples, a second PCR reaction was performed to amplify the signals. The fluorescence-labeled PCR products were then run on a sequencing gel and analyzed on an automated DNA sequencer using GeneScan software (Perkin-Elmer). To determine the significance of the spectratype data, 3 separate B6-control spectratype experiments (each using 2-3 mice) were performed to obtain a mean ± SD for the area represented by each peak of each Vβ spectratype. Experimental spectratypes were considered significantly skewed if a dominant peak exceeded the mean of control B6 mice by >4 SD ($P \leq .025$).

In Vivo Analysis for GVL Potential and GVHD

Syngeneic (B6 → B6) and allogeneic (B6 → B6D2) BMTs were performed to determine the antileukemia potential of Vβ-enriched T-cell populations. B6 recipients, lethally irradiated with 9.5 Gy (136 cGy/min) from a ^{137}Cs source (Mark-1 Model 68 gamma irradiator; J.L. Shephard, San Fernando, CA) and B6D2 recipients, lethally irradiated with split-dose 13 Gy, were injected 4 to 6 hours later with a B6 donor inoculum of 2×10^6 naive ATBM cells alone or in combination with various numbers of MMB3.19-primed T-cell populations. Donor T cells were either unfractionated, enriched for the CD4⁺ subset, or enriched for selected Vβ-expressing T cells and were from mice that had been presensitized 2 to 3 weeks earlier with irradiated (40 Gy) MMB3.19 cells (5×10^6, intraperitoneally [IP]). Recipients were challenged 1 day later with MMB3.19 cells (7.5×10^4 or 2×10^4, IP in PBS). Mice were monitored daily for morbidity and mortality and weighed 2 times per week until the termination of experiments. Median survival times (MSTs) were calculated, and the nonparametric Wilcoxon 2-sample rank test was used for statistical comparisons of survival curves.

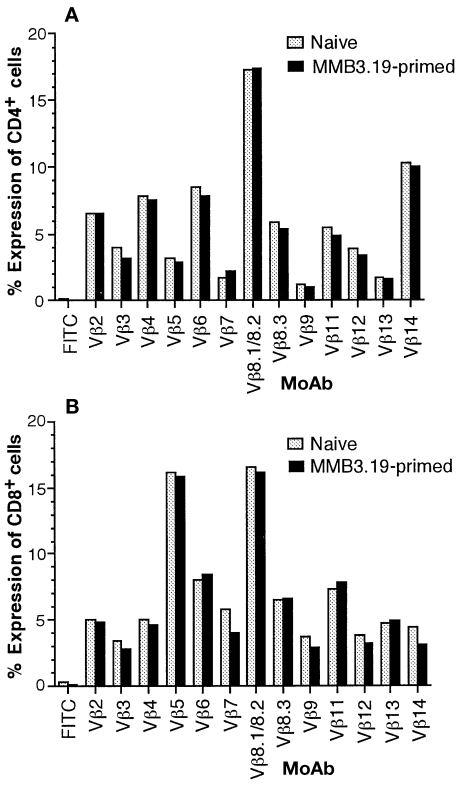

Figure 1. Flow cytometric analyses of donor MMB3.19-primed or naive CD4⁺ and CD8⁺ T cells. Donor CD4⁺ and CD8⁺ populations were isolated from either naive B6 mice (▨) or from mice presensitized 2 weeks prior with irradiated (40 Gy) MMB3.19 (■). Cells were stained with appropriate fluorescein isothiocyanate (FITC)-labeled Vβ-specific monoclonal antibodies (MoAbs). Results are displayed as percentage of total CD4⁺ (A) or CD8⁺ (B) T cells and represent data of pooled cells from 5 mice for each group.

RESULTS

Representation of Vβ Families in MMB3.19-Primed Donor T-Cell Populations

In previous studies using the MMB3.19 myeloid leukemia model, it was demonstrated that MMB3.19-primed donor CD4⁺ or unfractionated T cells were capable of mediating potent in vivo GVL responses following syngeneic B6 → B6 bone marrow transplantation [17,27,35]. The first step in the current investigation, therefore, was to analyze the TCR Vβ representation of the T-cell repertoire in the leukemia-primed donor mice. CD4⁺ or CD8⁺ T cells were harvested from either naive or MMB3.19-primed mice and flow cytometric analysis was performed using available Vβ-specific MoAbs. This analysis revealed that, although the representation of different Vβ families varied widely within subsets (from 1% to 18% of the total CD4⁺ or CD8⁺ populations), there were no obvious differences in the relative representation of Vβ families in naive mice compared with MMB3.19-primed mice (Figure 1A and B). This finding

suggests that any expansion of leukemia-reactive clonotypes did not significantly alter the overall percentage of the corresponding Vβ families.

TCR CDR3-Size Spectratype Analysis of Leukemia-Reactive and Alloreactive T-Cell Populations

Because leukemia-specific responses are possible in autologous and syngeneic transplantation [40-43] and in allogeneic transplantation without overt GVHD [44], an experimental system was used whereby tumor reactivity and alloreactivity could be clearly differentiated without the confounding effects of alloantigens expressed on tumor cells. To this end, studies were conducted in both a syngeneic antileukemia system (B6 anti-MMB3.19) and a haploidentical transplantation system [B6($H2^b$) → B6D2($H2^{b/d}$)]. To specifically identify leukemia-reactive and alloreactive subpopulations of $CD4^+$ and $CD8^+$ T cells, TCR CDR3-size spectratype analyses were performed on MMB3.19-primed LN or splenic T cells from B6D2 transplantation recipients and compared with control naive-B6 T-cell spectratypes. Both leukemia-primed and alloreactive $CD4^+$ and $CD8^+$ T-cell populations exhibited oligoclonal responses, with both unique and overlapping Vβ family involvement (Table). In the $CD4^+$ anti-MMB3.19 response, unique expansions (relative to the alloreactive response) were detected in the Vβ2, 7, 13, and 15 families. In the $CD8^+$ response, which was more heterogeneous than the $CD4^+$ pattern, unique expansions were observed in the Vβ4, 6, 7, 8, 10, 12, and 15 families.

*Summary of T-Cell Receptor Vβ Complementarity-Determining Region 3–Size Spectratype Analyses**

	B6 anti-B6D2		B6 anti-MMB3.19	
Vβ Family	$CD4^+$	$CD8^+$	$CD4^+$	$CD8^+$
1	–	–	–	+/–
2	–	+	+	–
3	+/–	+	–	+/–
4	+/–	–	NS	+
5	+	–	–	–
6	–	–	–	+
7	+/–†	–	+	+
8	+	+/–	+	+
9	NS	+	NS	+
10	+	+/–	+	+
11	–	+	+/–	+
12	+	–	–	+
13	+/–	+	+	–
14	+	+	+	+
15	–	+/–	+	+
16	+	–	+/–	–
18	–	+	–	+
20	–	–	NS	–

*All spectratype experiments were performed twice. + indicates significantly skewed band(s) were detected compared with a control naive C57BL/6 (B6) T-cell population; –, no skewed bands; +/–, skewing of band(s) in 1 experiment but not the other; NS, no spectratype was observed in either experiment.

†Skewed band in B6 anti-(B6×DBA/2)F1 (B6D2) Vβ7 $CD4^+$ was different from that in B6 anti-MMB3.19 Vβ7 $CD4^+$ spectratype.

Using a Syngeneic BMT Model to Investigate GVL Potential of Leukemia-Reactive Vβ Families

To determine whether CDR3-size spectratyping could identify antileukemia populations of T cells capable of mediating an in vivo GVL effect, we administered selected Vβ families of unfractionated (containing both $CD4^+$ and $CD8^+$) B6 T cells based on the anti-MMB3.19 tumor-skewing profiles. The $V\beta7^+$ T-cell family was chosen because it exhibited highly skewed spectratypes for both $CD4^+$ and $CD8^+$ T-cell subsets (Figure 2D and E). In addition, $V\beta11^+$ and $V\beta13^+$ families were chosen based on their skewed profiles in $CD8^+$ and $CD4^+$ T-cell populations, respectively (Figure 2A and B). Administration of B6 ATBM and 1×10^6 MMB3.19-primed Vβ7,11,13-enriched T cells to irradiated B6 recipients conferred potent GVL responses with 88% long-term survivors (MST, >44 days; $P \leq .01$) compared with the early fatality of control MMB3.19-challenged mice receiving only ATBM cells (MST, 21 days; Figure 3). This level of protection was not significantly different ($P > .30$) from that observed in a group receiving an equivalent number (1×10^6) of unfractionated MMB3.19-primed B6 T cells, which demonstrates that the protective GVL effect could be mediated by a population of T cells enriched (>70%) for the $V\beta7^+,11^+,13^+$ families. A repeat experiment using half the number of Vβ7,11,13-enriched T cells (5×10^5) similarly provided long-term GVL activity (80% survival of >58 days compared to 60% receiving equal numbers of unfractionated MMB3.19-primed T cells; $P > .66$; data not shown).

To more specifically address the GVL potential of individual Vβ families, 2 of the B6 $CD4^+$ Vβ families that exhibited highly skewed antileukemia spectratypes, Vβ7 and Vβ13 (Figure 2B and D), were each selected for transplantation into syngeneic recipients. Upon MMB3.19 leukemia challenge, B6 recipients of B6 ATBM alone or with 2×10^5 Vβ13-enriched (>60%) $CD4^+$ T cells displayed similar mortality rates (MST, 17.5 or 18 days for groups receiving ATBM alone or with Vβ13-enriched $CD4^+$ T cells; $P \geq .65$) (Figure 4A) and, therefore, failed to display any effective GVL response. In contrast, transfer of 1×10^5 Vβ7-enriched (>50%) B6 $CD4^+$ T cells significantly extended survival (MST, 22 days) of MMB3.19-challenged recipients compared with those receiving ATBM alone or in combination with 1×10^5 Vβ7-depleted B6 $CD4^+$ T cells ($P \leq .05$). An inoculum of 2×10^6 unfractionated MMB3.19-primed $CD4^+$ T cells (equivalent to a $CD4^+$ T-cell population that would contain at least 5×10^4 $V\beta7^+$ cells) was used as a reference point for the GVL effect and provided a marginally better GVL response (MST, 24 days versus 22 days for the $V\beta7^+$ group; $P > 0.05$) than that observed in recipients of the Vβ7-enriched $CD4^+$ T cells. These results suggest that there are other GVL participants in addition to the $V\beta7^+$ population, but the $V\beta7^+$ responders certainly formed an important component of the CD4-mediated GVL response. Hypothetically, the $V\beta13^+$ population could still be involved in the antileukemia response by, for example, contributing to the level of essential inflammatory cytokines, even though on their own they were ineffectual mediators of GVL activity.

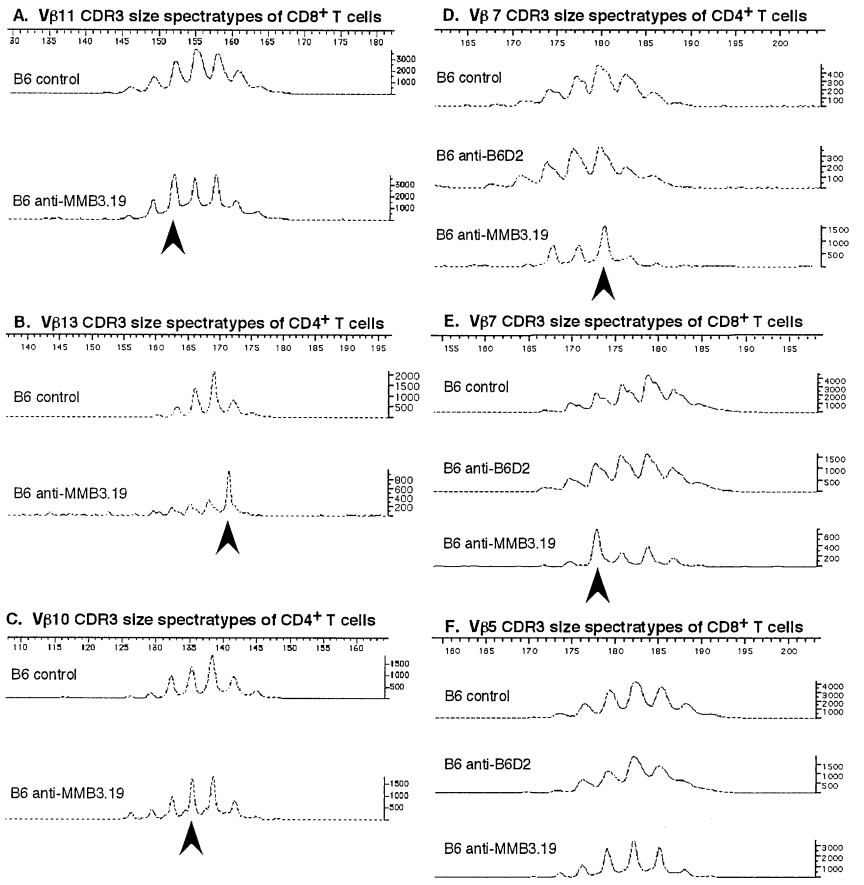

Figure 2. Selected histograms of T-cell receptor complementarity-determining region 3 (CDR3)-size spectratype analyses of $CD4^+$ and $CD8^+$ T-cell populations. Histograms of $V\beta11^+$ $CD8^+$ (A) and $V\beta13^+$ $CD4^+$ (B) T cells from naive or MMB3.19-presensitized mice with significant skewing (arrow) in the latter populations. C, Histograms of $V\beta10^+$ $CD4^+$ T cells from naive or MMB3.19-presensitized mice as an example of a statistically, but not obviously, skewed Vβ family (arrow). Histograms of $V\beta7^+$ $CD4^+$ (D) and $CD8^+$ (E) T cells from naive C57BL/6 (B6), transplant-receiving B6D2, or MMB3.19-presensitized B6 mice with significant skewing (arrow) in the leukemia-primed population, but not in the B6 anti-B6D2 population. F, Histograms of $V\beta5^+$ $CD8^+$ T cells demonstrating no significant skewing in either leukemia-primed or alloreactive T cells.

Separation of GVL Effects From GVH Effects in an Allogeneic BMT Model Using Leukemia-Reactive Vβ Families

After it was demonstrated that CDR3-size spectratyping could identify anti-MMB3.19 leukemia populations of T cells that can mediate in vivo GVL effects in syngeneic recipients, the next important step was to test the hypothesis that selective administration of these tumor-reactive Vβ families in a semiallogeneic situation could allow development of GVL responses with minimal induction of GVHD. To approach this experimentally, 2 T-cell Vβ families (Vβ6 and Vβ7) were selected by spectratyping on the basis of their detected antileukemia reactivity without overt B6 anti-B6D2 ($H2^{b/d}$) alloreactivity in either the CD4 or CD8 subsets (Table). In addition, in the case of the Vβ7 population, the GVL potential in the syngeneic model, described above, had already been demonstrated (Figure 4B). It was also hypothesized that optimum activity of the $V\beta6^+$CD8 T-cell antitumor population, detected by the spectratype analysis, would be enhanced by the presence of CD4-derived help. Positively-selected $V\beta6^+7^+$ cells (7×10^5 cells) from MMB3.19-primed B6 mice were transferred along with ATBM to lethally irradiated haploidentical B6D2 mice that were subsequently challenged with MMB3.19 cells. Recipients of Vβ6,7-enriched (80%) T cells exhibited significantly prolonged survival times ($P < .05$) over those receiving an equal number of MMB3.19-primed unfractionated T cells (Figure 5A). It is important to note that because a sublethal dose of donor T cells was used, there were no deaths in corresponding groups that were not challenged with the MMB3.19 leukemia. Therefore, mortality was due either to leukemia burden alone or to a combination of leukemia and GVH effects, but not to GVHD alone. Weight loss was the parameter used to determine the severity of GVHD in those groups that did not receive MMB3.19. B6D2 recipients of Vβ6,7-enriched T cells experienced significantly less weight loss than those receiving the unfractionated T cells ($P < .05$ from day 35 onward), although some disease effects were still evident in comparison with the ATBM control group (Figure 5B). These data indicate that selective administration of leukemia-reactive Vβ families can provide a significant GVL effect while decreasing the severity of GVHD.

To further reduce GVHD, the GVL potential of B6 $V\beta7^+$ $CD4^+$ T cells alone was investigated in the semiallogeneic

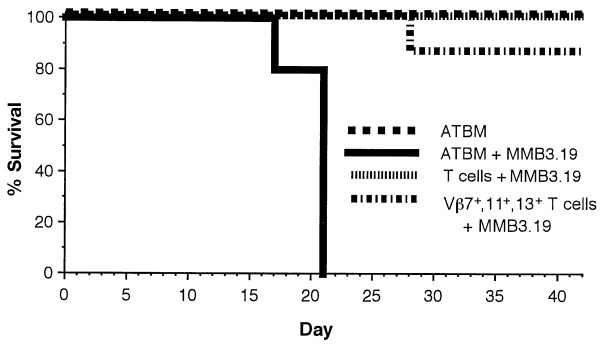

Figure 3. Vβ7,11,13-enriched donor T cells provide a graft-versus-leukemia effect in a syngeneic bone marrow transplantation model. Lethally irradiated (9.5 Gy) B6 mice received 2×10^6 B6 anti-Thy-1 monoclonal antibody–treated (T cell–depleted) bone marrow (ATBM) cells and either 1×10^6 unfractionated MMB3.19-primed B6 T cells or 1×10^6 Vβ7,11,13-enriched (>70%) T cells. Aside from an ATBM control group, all mice were challenged on day 1 with 7.5×10^4 MMB3.19 cells administered intraperitoneally and monitored for survival. For all groups, n = 5, except for the group receiving $V\beta7^+11^+13^+$ T cells (n = 8). A repeat experiment using half the number of T cells yielded similar results.

transplantation setting. To enhance potential GVL effects, the recipient mice were challenged with a lower dosage of 2×10^4 MMB3.19 cells. Leukemia-challenged B6D2 recipients of 2.5×10^5 MMB3.19-primed Vβ7-enriched (82%) $CD4^+$ T cells exhibited significantly extended survival times (MST, 66 days) compared with recipients of ATBM alone or of unfractionated MMB3.19-primed $CD4^+$ T cells (both with MST of <33 days; $P < .05$) (Figure 6A). An important observation was that B6D2 recipients of $V\beta7^+$-enriched $CD4^+$ T cells experienced little, if any, weight loss compared with recipients of ATBM alone (Figure 6B). In contrast, recipients of 2.5×10^5 unfractionated $CD4^+$ donor T cells experienced a detectable level of GVHD, as evidenced by significant weight loss ($P < .05$ from day 25 onward, except for day 40) (Figure 6B). These results support the hypothesis that selective administration of leukemia-reactive Vβ families can provide a significant GVL effect without corresponding GVHD.

DISCUSSION

Using both syngeneic and semiallogeneic murine HSCT models, we have found that transplantation of donor leukemia-reactive T-cell subpopulations, identified by TCR Vβ CDR3-size spectratyping, can provide potent GVL effects. An important result was that in the semiallogeneic model this GVL effect coincided with the lack of significant antihost GVHD development. To clarify our tumor model system, in a semiallogeneic haploidentical situation of either $P \rightarrow F_1$ ($a \rightarrow a \times b$ haplotypes) or $(a \times b) \rightarrow (a \times c)$ combinations (most representative of parent → child clinical transplantations), donor cells will be capable of recognizing the host alloantigens expressed by the incompatible MHC molecules on the tumor cells, as well as tumor-specific antigens presented on compatible syngeneic MHC molecules shared by the donor. We selected our model as the optimum one to clearly address the hypothesis of whether these syngeneic antitumor responses can be selected for, based on Vβ spectratype analysis, while avoiding the alloreactive responses. We chose the B6 → $(B6D2)F_1$ model because it avoids the complication of marrow rejection and allows us to focus only on the alloreactive antihost and antitumor responses. In this model, we challenged with the B6-origin MMB3.19 leukemia cells, which were completely syngeneic to the donor. We used this cell type because a myeloid tumor of the F_1 type did not exist and because we wanted to focus on the "true" antitumor antigen response, uncomplicated by the potential alloreactive component against the tumor cells. Theoretically, the Vβ repertoire approach will not work in a completely allogeneic transplantation situation, because allogeneic donor cells

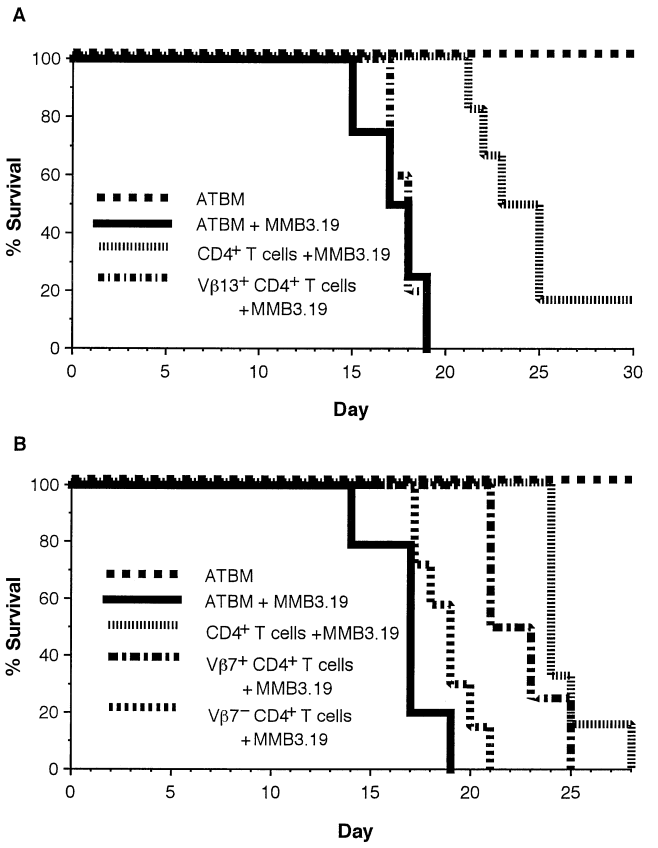

Figure 4. Vβ7-enriched $CD4^+$ T cells, but not $V\beta13^+$ $CD4^+$ T cells, provide a graft-versus-leukemia (GVL) effect in a syngeneic bone marrow transplantation model. Lethally irradiated (9.5 Gy) B6 mice were injected with 2×10^6 anti-Thy-1 monoclonal antibody–treated (T cell–depleted) bone marrow (ATBM) cells and MMB3.19-primed $CD4^+$ T cells that were either (A) Vβ13-enriched (60%; 2×10^5) or (B) Vβ7-enriched (>50%; 1×10^5). As a reference point for GVL activity, 2×10^6 unfractionated MMB3.19-primed $CD4^+$ T cells were also administered in each experiment. Aside from an ATBM control group, all mice were challenged on day 1 with 7.5×10^4 MMB3.19 cells administered intraperitoneally and monitored for survival. Results are representative of 2 similar experiments using Vβ13-enriched $CD4^+$ T cells and 3 similar experiments using various doses of Vβ7-enriched $CD4^+$ T cells.

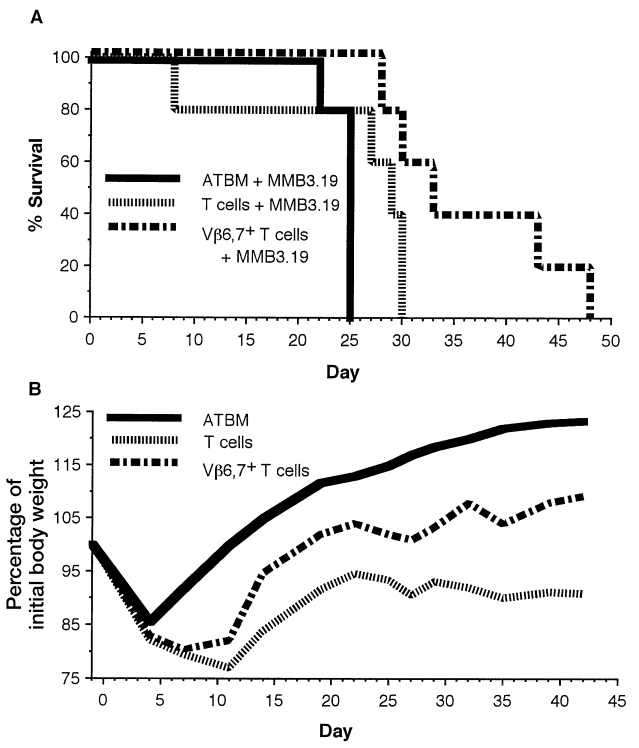

Figure 5. Vβ6,7-enriched T cells mediate a graft-versus-leukemia effect with minimization of graft-versus-host disease (GVHD) in an allogeneic bone marrow transplantation (BMT) model. Lethally irradiated B6D2 mice were injected with 2×10^6 B6 10^6 anti-Thy-1 monoclonal antibody–treated (T cell–depleted) bone marrow (ATBM) cells and either 7×10^5 MMB3.19-primed Vβ6,7-enriched (80%) T cells or an equal number of unfractionated T cells. A, Survival curves for groups receiving MMB3.19 challenge (7.5×10^4; intraperitoneally) 1 day post-BMT. B, Percentage of initial body weight for groups not receiving leukemia challenge and therefore GVHD-associated. For all groups, n = 5, and the results are representative of 2 similar experiments.

would be able to recognize only alloantigens (incompatible MHC) on the tumor cells. Thus, we believe that the semiallogeneic model system is the most suitable for addressing the potential of the Vβ family GVL selection approach.

TCR Vβ CDR3-size spectratyping has been used to analyze and track immune responses to known antigens [45-47] as well as unknown antigens [28,33,34,48]. Because Vβ spectratyping allows for identification of responding T-cell populations without intimate knowledge of the target antigens, it has potential clinical application, especially in the area of HSCT for leukemia. Selecting dominant tumor-reactive Vβ families from donor T cell–subset populations may allow for an effective GVL response while decreasing the risk of severe GVHD development. Operationally, alloreactive responses often generate heterogeneous Vβ family involvement, as exemplified in the B6 anti-B6D2 response (Table), in which two thirds of all Vβ families tested exhibited skewing for the entire T-cell population. However, this response repertoire is narrowed when one focuses on individual $CD4^+$ and $CD8^+$ T-cell subsets, in which only about one third of the repertoire was skewed in either case. This more limited Vβ family involvement leaves a greater window for finding unique antileukemia Vβ family responses within the subsets. In the clinical setting, these unique antileukemia Vβ responses could potentially be exploited by using selection techniques (depending upon availability of Vβ-specific MoAbs) to provide these potential GVL responding cells in conjunction with HSCT, either at the time of initial transplant or as delayed infusions.

There is extensive controversy over whether clinical GVL responses can actually be separated from GVHD alloreactivity directed to either MHC or minor histocompatibility host antigens. Several studies support the argument that a significant portion of the GVL response is due to alloreactivity [49-54]. On the other hand, leukemia-specific responses are possible, as demonstrated by in vitro cytotoxic T-lymphocyte studies targeting proteinase 3 [32] and bcr-abl junctional peptides [29-31] and by observations of GVL effects in autologous and syngeneic transplantations

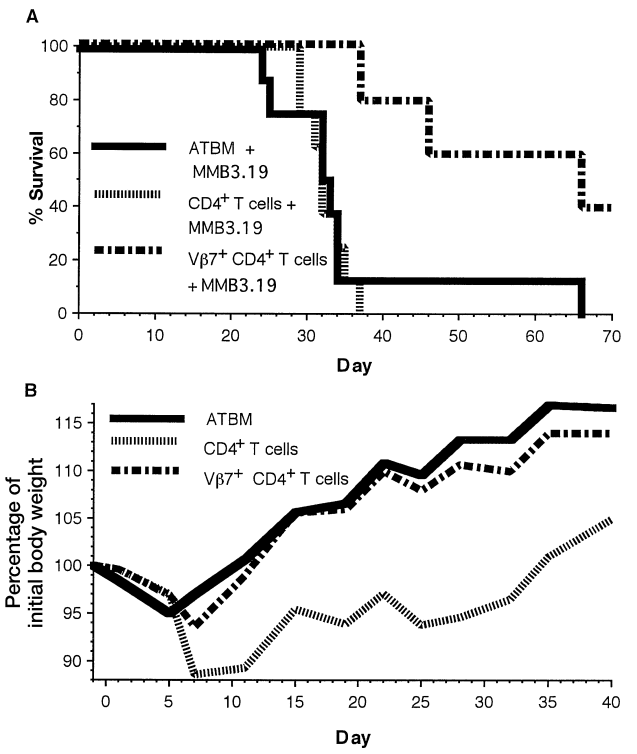

Figure 6. Vβ7-enriched $CD4^+$ T cells mediate a graft-versus-leukemia response with minimal graft-versus-host disease (GVHD) induction in an allogeneic bone marrow transplantation (BMT) model. Lethally irradiated B6D2 mice were injected with 2×10^6 B6 anti-Thy-1 monoclonal antibody–treated (T cell–depleted) bone marrow (ATBM) cells and 2.5×10^5 MMB3.19-primed Vβ7-enriched (82%) B6 $CD4^+$ T cells or an equal number of unfractionated $CD4^+$ T cells. A, Survival curves for groups receiving MMB3.19 challenge (2×10^4 cells administered intraperitoneally) 1 day post-BMT. B, Percentage of initial body weight for groups not receiving leukemia challenge and indicative of GVHD. Groups contained 4 to 8 mice and were representative of results from 2 similar experiments.

[41-43]. Certainly, the availability of leukemia-specific antigens will vary depending on the type and stage of the tumor involved, the individual patient's MHC molecules required for presentation, and the repertoire of the donor's responding T cells. However, the potential for assessing leukemia-specific responses, if they can be induced, is afforded by Vβ spectratype analysis, even without prior knowledge of the antigens involved. Importantly, Jiang et al. [55] have identified leukemia-specific T-cell clones that show different preferential Vβ usage than do clones reactive to autologous phytohemagglutinin blasts, suggesting that leukemia-specific populations may be separable by Vβ expression.

In the allogeneic transplants using leukemia-reactive Vβ6,7-enriched T cells (Figure 5), there was still a detectable level of GVHD indicated by comparative weight loss. This activity could be due either to residual contaminating alloreactive cells (from other Vβ families) or to the possibility that the CDR3-spectratype analysis did not detect low-level alloreactivity within the Vβ6 and 7 families. Regarding the purity of the donor cell preparations, it was technically difficult to achieve a high level of purity of Vβ families that normally represented a small percentage of the overall initial T-cell population. For example, $V\beta13^+$ $CD4^+$ T cells represented about 1% to 2% of the total $CD4^+$ population (Figure 1), so enrichment to 60% to 70% of the population (Figure 4A) was considered to be substantial. However, such a level of purity still allows for the possibility of contaminating alloreactive cells in the transplant, and there is certainly room for improvement in this approach. Nevertheless, the absolute number of potentially alloreactive cells is diminished in these leukemia-reactive Vβ-enriched cell preparations in relation to those containing unfractionated T cells and would be expected to result in a correspondingly lower level of GVHD. Importantly, administration of a single leukemia-reactive Vβ family of $CD4^+$ T cells eliminated detectable GVHD (Figure 6B), indicating that this method of identification and enrichment of leukemia-reactive populations could effectively lead to GVL activity without GVHD.

Many issues will have to be addressed for this identification and separation approach to be effective in a clinical setting. One of these issues is the availability of MoAbs specific for many of the human Vβ regions. These antibodies must be developed before the Vβ enrichment process can be optimally performed. Another important clinical consideration relates to potential increased graft failure of selected Vβ-enriched donor populations, if administered at the time of HSCT. This problem may be obviated by more intensive preconditioning regimens or by increased numbers of donor stem cells. Alternatively, the use of Vβ-selected populations could be reserved for the donor-lymphocyte infusion (DLI) setting, so that engraftment would not be an issue at that time. Another clinically related obstacle involves procurement of leukemia samples before debulking treatments are initiated. Potential recipients would have to be selected early in a treatment protocol to obtain tumor samples for in vitro restimulation experiments. In addition, in vitro restimulation of potential antileukemia populations will require defining the optimal in vitro conditions needed to identify and expand those donor T-cell populations that demonstrate a preferential antileukemia response. Furthermore, if long-term culturing is required, decreased reactivity of long-term T-cell cultures will have to be addressed. Although each of these obstacles will require significant effort to address, each is ultimately solvable.

Because the approach we describe here requires identification of leukemia-reactive and alloreactive Vβ families by spectratype analysis, it would be particularly well-suited clinically in a DLI setting, as mentioned above. The time lapse between initial stem cell transplantation and DLI would allow for identification, selection, and possibly expansion of leukemia-restricted donor T cells. Such an approach should enhance the benefits of DLI, permitting a beneficial GVL effect while reducing the incidence and severity of GVHD.

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